

**United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program**

SOP No.: MDP-MTH-01A		Page 1 of 7
Title: Enumeration of <i>Escherichia coli</i> in Produce Samples by TEMPO® EC (<i>E. coli</i>) System		
Revision: Original	Replaces: None	Effective: 05/01/07

1. Purpose & Principle

The TEMPO® EC (*E. coli*) is an automated system designed to provide a standard procedure based on the Most Probable Number (MPN) for enumeration of *Escherichia coli* (*E. coli*) in fresh fruit and vegetables. In TEMPO system, the MPN test is presented as a card carrying 3 sets of 16 wells (48 wells in total). The identification is based on the presence of a specific enzyme, β -D-glucuronidase, in over 95% of the *E. coli* strains (including non-gas producing). This enzyme cleaves the substrate, 4-methylumbelliferyl β -D-glucuronidase (MUG), provided in the culture medium. The resulting compound, 4-methylumbelliferone (MU), fluoresces upon exposure to ultraviolet (UV) light. This fluorescent signal is detected by a TEMPO reader and based on the MPN method (depending on the number and type of positive wells) the TEMPO system calculates the number of *E. coli* present in the original sample.

Scope

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

3. Outline of Procedures

Equipment and Materials	5.1
Media and Reagents	5.2
List of Controls	5.3
TEMPO® EC (<i>E. coli</i>) based MPN Method	5.4
Calculating MPN and Reporting Data	5.5

4. References

- 4.1. TEMPO® EC (*E. coli*) REF 80 004 dated 10/2006, provided by bioMerieux® SA
 - 4.2. TEMPO® QC REF 80 000 dated 04/2004, provided by bioMerieux® SA, for the verification of TEMPO Reader measurement performance
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- 4.3. International Standard ISO 16649-2 (May 2001) – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β -glucuronidase-positive *Escherichia coli*. Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl β -D-glucuronide.
- 4.4. Peter Feng, Stephen D. Weagant, Michael A. Grant. 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria. Chapter 4. FDA. BAM. <http://www.cfsan.fda.gov/~ebam/bam-4.html> (last accessed 11/10/05).
- 4.5. Peter Feng and Stephen D. Weagant. 2002. Diarrheagenic *Escherichia coli*. Chapter 4A. FDA. BAM. <http://www.cfsan.fda.gov/~ebam/bam-4a.html> (last accessed 11/10/05).
- 4.6. SOP MDP-LABOP-02, Sample Receipt, Elution, Preenrichment, and DNA Extraction.
- 4.7. SOP MDP-DATA-01, Data Entry, Record Keeping, and Results Reporting
- 4.8. SOP MDP-QA-03, Quality Assurance Controls
- 4.9. Evaluation of Universal Pre-enrichment Broth (UPB) as a Wash Buffer for Produce Commodities. Final study report, Division of Consolidated Laboratory Services (DCLS), Department of General Services, Commonwealth of Virginia. October 2005.

5. Specific Procedures

5.1. Equipment and Materials

- 5.1.1. TEMPO® system
- 5.1.2. Incubator, 35 \pm 2°C
- 5.1.3. Serological pipets, sterile
- 5.1.4. Vortex mixer
- 5.1.5. TEMPO incubation rack
- 5.1.6. Dispensers
- 5.1.7. TEMPO filler rack
- 5.1.8. Tempo filter bags, sterile

5.2. Media and Reagents

- 5.2.1. TEMPO® EC cards
 - 5.2.2. TEMPO® EC culture medium vials
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5.2.3. Sterile water

5.2.4. TEMPO® QC kit

5.2.5. Eluent: Universal Preenrichment Broth (UPB) from Difco or equivalent, supplemented with 0.1% Tween 80 (UPBt)

5.2.6. Control culture broth: UPBt or any sterile non-specific rich media

5.3. List of controls (specific strains are listed in SOP MDP-QA-03)

5.3.1. Negative culture control: 1 mL overnight culture, diluted appropriately (e.g. ~100 CFU/mL) in UPBt or any sterile non-specific rich media

5.3.2. Positive culture control: 1 mL overnight culture, diluted appropriately (e.g. ~100 CFU/mL) in UPBt or any sterile non-specific rich media

5.3.3. Positive produce culture control (refer to SOP MDP-LABOP-02): 1 mL spiked culture

5.3.4. Media controls: UPBt

5.3.5. Carry all controls through this entire procedure. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03. The expected results of QC control cultures after a minimum of 24 hours of incubation should conform to those shown below:

QC Culture	Enumeration Range
Uninoculated media	Low threshold < 1
Positive Control	Higher than low threshold
Negative Control	Low threshold < 1
Produce Control	Higher than low threshold

5.4. TEMPO® EC Method

Follow the manufacturer's instructions for handling, storage and use of the TEMPO cards and reagents vials, reconstitution of culture reagents, and TEMPO cards setup and filling.

5.4.1. Remove TEMPO EC vials and cards and allow them to reach room temperature.

5.4.2. For samples, transfer 10 to 25 mL of each sample wash from MDP-LABOP-02 to a sterile TEMPO filter bag.

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5.4.3. Refer to manufacturer's instructions for TEMPO card setup instructions.

5.4.4. Reconstitute the contents of each vial as follows:

5.4.4.1. Dispense 3 mL of sterile water into a TEMPO EC vial

5.4.4.2. Transfer 1 mL of wash eluate from section 5.4.2 into one vial and mix by vortexing.

5.4.4.3. Transfer 1 mL of each of the controls from section 5.3 into an individual vial and mix by vortexing.

5.4.5. Follow the manufacturer's instructions for card filling, incubation and reading the cards. Enter appropriate dilution level in the computer.

Note: TEMPO dilution factor is calculated based on the volume of the wash (UPBt) used for a given commodity and the 4 fold dilution of wash sample during the reconstitution of TEMPO EC culture medium vials.

5.5. Results and Interpretation

5.5.1. The results are automatically read and analyzed by the computer. The enumeration number represents CFU per milliliter of the eluate. Report CFU/mL, as displayed by the computer, for each sample. Normalization of MPN data per gram of the produce will be performed by MPO.

5.5.2. Report MPN for *E. coli* according to SOP MDP-DATA-01. MPN values shall be reported only in RDE; MDP-MTH-01 Attachment 1 (Preliminary and Final Reporting Results) shall not be completed for positive MPN values.

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TEMPO Dilutions and Enumeration Ranges

TEMPO Dilution		Enumeration Range	
		Low Threshold	High Threshold
1 /	1	1	1,200
	2	1	2,500
	4	1	4,900
	10	3	12,000
	20	5	25,000
	25	7	31,000
	30	8	37,000
	35	9	43,000
	40	10	49,000
	50	13	62,000
	70	18	86,000
	100	25	120,000

Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.

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- Established procedures for use of TEMPO® EC system for enumeration of *E. coli* in fresh fruit and vegetables